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Protocol: Hemocytometer Cell Counting

Application:
Manual counting of cell suspensions

Procedure:
1. Aliquot 100 μL of 0.4% Trypan Blue solution into appropriately labeled tube.

2. Aliquot 100 μL of pre-mixed cell suspension into the appropriately labeled tube from Step 1.
   - a 1:1 ratio of Trypan Blue to cell suspension (equals 2-fold dilution)[1].

3. Vortex briefly (touch-spin if using 1.5 mL microfuge tubes to bring contents down from lid).

4. Pipette 10 μL of Trypan Blue-cell suspension into Hemocytometer chamber, between chamber notch and glass coverslip (**Figure 1A**, black arrow).

5. Count cells in boxes 1-4 (**Figure 1B**, red boxes)[2,3].

6. Calculate (A) cells/mL and (B) total cells in your sample using the formulas below:

$$\text{(A) Cells/mL} = \left(\frac{\text{total cells counted}}{\text{number of boxes counted}}\right) \times \text{dilution factor} \times 10{,}000$$

$$\text{(B) Total cells in sample} = \text{cells/mL x total sample volume}$$

Example: (100 cells counted / 4 boxes) x 2 x 10,000 = 500,000 (or 5 x 10^5) cells/mL
5 x 10^5 cells/mL x 10 mL sample volume = 5,000,000 (or 5 x 10^6) total cells in sample

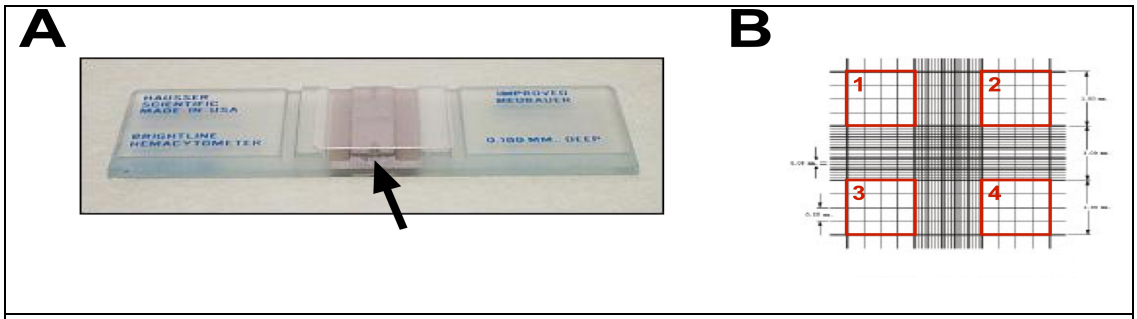

Figure 1: Hemocytometer overview. (A) View of Hemocytometer with glass coverslip. Black arrow denotes notch used for loading cell samples. (B) Illustration of Hemocytometer counting area. In general, boxes 1-4 (red) are used to count cells.

[1] Trypan Blue-cell suspension dilution may change based upon predicted cell concentration.
[2] Aim to count ≥100 cells. If counting ≥100 cells/box, then less boxes can be counted assuming equal distribution of cells across the Hemocytometer chamber.
[3] To avoid overestimation of cell numbers, include cells that lie on the top and right edges of each box. Do not include cells along the bottom and left edges.